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(71) Applicant (for all designated States except US): **THIRD WAVE TECHNOLOGIES, INC.** [US/US]; 502 South Rosa Road, Madison, WI 53719 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ALLAWI, Hatim** [JO/US]; 33 Oakbridge Court #21, Madison, WI 53717 (US). **BARTHOLOMAY, Christian, Tor** [US/US]; 1913 Reetz Road, Madison, WI 53711 (US). **CHEHAK, Lu-Anne** [US/US]; 3211 Vold Court, Apartment 8, Janesville, WI 53546 (US). **CURTIS, Michelle, L.** [US/US]; 2740 Nadene Road, Cottage Grove, WI 53527 (US). **EIS, Peggy, S.** [US/US]; 5781 Pembroke Drive, Madison, WI 53711 (US). **HALL, Jeff, G.** [US/US]; 6305 Dylan Drive, Madison, WI 53719 (US). **IP, Hon, S.** [CA/US]; 6310 Pilgrim Road, Madison, WI 53711 (US). **KAISER, Michael** [US/US]; 2901 Shefford Drive, Madison, WI 53719 (US). **KWIATKOWSKI, Robert, W., Jr.** [US/US]; 506 Azurene Lane, Verona, WI 53593 (US). **LUKOWIAK, Andrew, A.** [US/US]; 2254 High Ridge Trail, Madison, WI 53713 (US). **LYAMICHEV, Victor** [RU/US]; 2523 Carriedale Court, Madison, WI 53711 (US). **MA, WuPo** [CN/US]; 1110 N. Westfield Road, Madison, WI 53717 (US). **OLSON-MUNOZ, Marilyn,**

**C.** [US/US]; 5878 Tree Line Drive, Madison, WI 53711 (US). **OLSON, Sarah, M.** [US/US]; 2968 Thinnes Street, Cross Plains, WI 53528 (US). **SCHAEFER, James, J.** [US/US]; 4369 Crawford Drive, Madison, WI 53711 (US). **SKRZYPCZYNSKI, Zbigniew** [PL/US]; 881 Orchid Street, Verona, WI 53953 (US). **TAKOVA, Tsetska, Y.** [US/US]; 9 Oak Glen Court, Madison, WI 53717 (US). **VEDVIK, Kevin, L.** [US/US]; 1302 Monica Lane, Madison, WI 53704 (US). **LYAMICHEV, Natalie, E.** [RU/US]; 2523 Carriedale Court, Madison, WI 53711 (US). **NERI, Bruce, P.** [US/US]; 5714 Kilkenny Place, Madison, WI 53711 (US).

(74) Agents: **CASIMIR, David, A.** et al.; Medlen & Carroll, LLP, Suite 350, 101 Howard Street, San Francisco, CA 94105 (US).

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(54) Title: DETECTION OF RNA

(57) Abstract: The present invention provides novel cleavage agents and polymerases for the cleavage and modification of nucleic acid. The cleavage agents and polymerases find use, for example, for the detection and characterization of nucleic acid sequences and variations in nucleic acid sequences. In some embodiments, the 5' nuclease activity of a variety of enzymes is used to cleave a target-dependent cleavage structure, thereby indicating the presence of specific nucleic acid sequences or specific variations thereof.



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## A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
L,X	WO 98 42873 A (THIRD WAVE TECHNOLOGIES INC) 1 October 1998 (1998-10-01) L: Priority the whole document ----	1-107
X	WO 98 23774 A (THIRD WAVE TECH INC ;KAISER MICHAEL W (US); LYAMICHEV VICTOR I (US) 4 June 1998 (1998-06-04)  examples 41-43 ----	1,2, 9-11, 17-31, 38-40, 48-58
X	WO 97 27214 A (THIRD WAVE TECHNOLOGIES INC) 31 July 1997 (1997-07-31) the whole document ----- -/--	58-107

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Cupido, M

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/17086

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 892 058 A (HOFFMANN LA ROCHE) 20 January 1999 (1999-01-20)  page 6, line 11 -page 8, line 5 -----	1-6, 12-14, 16-23
X	US 5 972 603 A (TABOR STANLEY ET AL) 26 October 1999 (1999-10-26) example 12 -----	1-8, 12-23
X	US 5 837 450 A (BROW MARY ANN D ET AL) 17 November 1998 (1998-11-17) the whole document -----	58-107
X	US 6 001 567 A (OLIVE DAVID MICHAEL ET AL) 14 December 1999 (1999-12-14) cited in the application column 50 -column 55 -----	1-12
P,X	WO 01 01823 A (ODWALLA INC ;SMITH R E) 11 January 2001 (2001-01-11)  page 31 -page 35 -----	1-14, 18-38, 48-58
A	KAISER MICHAEL W ET AL: "A comparison of Eubacterial and Archaeal structure-specific 5'-exonucleases." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 30, 23 July 1999 (1999-07-23), pages 21387-21394, XP002185114 ISSN: 0021-9258 cited in the application the whole document -----	1-58
P,X	MA WU-PO ET AL: "RNA template-dependent 5' nuclease activity of Thermus aquaticus and Thermus thermophilus DNA polymerases." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 32, 11 August 2000 (2000-08-11), pages 24693-24700, XP002204059 ISSN: 0021-9258 the whole document -----	1-58

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/17086

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9842873	A	01-10-1998	US 5994069 A	30-11-1999
			AU 738849 B2	27-09-2001
			AU 6869098 A	20-10-1998
			EP 0994964 A1	26-04-2000
			JP 2001518805 T	16-10-2001
			WO 9842873 A1	01-10-1998
WO 9823774	A	04-06-1998	US 6090606 A	18-07-2000
			US 5843669 A	01-12-1998
			AU 731062 B2	22-03-2001
			AU 1836497 A	20-08-1997
			AU 737449 B2	23-08-2001
			AU 5589898 A	22-06-1998
			CA 2243353 A1	31-07-1997
			EP 0904286 A1	31-03-1999
			EP 0966542 A1	29-12-1999
			JP 2001526526 T	18-12-2001
			JP 2002515737 T	28-05-2002
			WO 9727214 A1	31-07-1997
			WO 9823774 A1	04-06-1998
			US 5994069 A	30-11-1999
WO 9727214	A	31-07-1997	US 6090606 A	18-07-2000
			US 5846717 A	08-12-1998
			US 6001567 A	14-12-1999
			US 5985557 A	16-11-1999
			US 6090543 A	18-07-2000
			AU 731062 B2	22-03-2001
			AU 1836497 A	20-08-1997
			AU 737449 B2	23-08-2001
			AU 5589898 A	22-06-1998
			CA 2243353 A1	31-07-1997
			EP 0904286 A1	31-03-1999
			EP 0966542 A1	29-12-1999
			JP 2001526526 T	18-12-2001
			JP 2002515737 T	28-05-2002
			WO 9727214 A1	31-07-1997
			WO 9823774 A1	04-06-1998
			US 5994069 A	30-11-1999
			US 6348314 B1	19-02-2002
			US 5843669 A	01-12-1998
EP 0892058	A	20-01-1999	CA 2240570 A1	09-01-1999
			EP 0892058 A2	20-01-1999
			JP 11089588 A	06-04-1999
			US 6228628 B1	08-05-2001
US 5972603	A	26-10-1999	WO 9729209 A1	14-08-1997
US 5837450	A	17-11-1998	US 5614402 A	25-03-1997
			US 5541311 A	30-07-1996
			US 5422253 A	06-06-1995
			US 5843654 A	01-12-1998
			US 5719028 A	17-02-1998
			US 5888780 A	30-03-1999
			AU 694736 B2	30-07-1998
			AU 7052094 A	03-01-1995
			CA 2163015 A1	22-12-1994

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/17086

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5837450	A	CA 2320666 A1	22-12-1994
		EP 0711361 A1	15-05-1996
		JP 8511684 T	10-12-1996
		WO 9429482 A1	22-12-1994
		US 5795763 A	18-08-1998
		US 5691142 A	25-11-1997
		AT 195141 T	15-08-2000
		DE 69329141 D1	07-09-2000
		DE 69329141 T2	11-01-2001
		DK 601834 T3	04-09-2000
		EP 0601834 A1	15-06-1994
		ES 2148208 T3	16-10-2000
		GR 3034654 T3	31-01-2001
		JP 6303975 A	01-11-1994
		PT 601834 T	31-01-2001
		US 5871911 A	16-02-1999
US 6001567	A 14-12-1999	US 5846717 A	08-12-1998
		AU 731062 B2	22-03-2001
		AU 1836497 A	20-08-1997
		CA 2243353 A1	31-07-1997
		EP 0904286 A1	31-03-1999
		JP 2002515737 T	28-05-2002
		WO 9727214 A1	31-07-1997
		US 6348314 B1	19-02-2002
		US 5985557 A	16-11-1999
		US 6090606 A	18-07-2000
		US 6090543 A	18-07-2000
		US 5994069 A	30-11-1999
		US 5843669 A	01-12-1998
WO 0101823	A 11-01-2001	AU 6804400 A	22-01-2001
		WO 0101823 A1	11-01-2001